

Evaluation of the impact of functional foods on the course of *Salmonella* infection in piglets

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Abstract

With the aim to improve growth of weaning piglets and to minimize incidence of intestinal diseases, the effect of a combination of functional foods able to stimulate the development of systemic and mucosal immune system and to modulate bacterial populations in the gut was evaluated. In this study, we assessed the impact of functional foods on the course of *Salmonella* infection in piglets. Piglets from different litters were weaned at 21 days of age and assigned to 1 of the 4 feed additives (8 litters per treatment) as follow: 1-control (CTRL), 2-antibiotic (ATB), 3-cocktail of functional foods (CFF), 4-bovine colostrum + cocktail of functional foods (COL-CFF). At 49 days of age (day 0), four piglets per litter were orally inoculated with 1×10^8 CFU of *Salmonella* Typhimurium. A clinical exam was done for each piglet twice a day. Fecal samples were taken to evaluate the *Salmonella* shedding before and post-infection (days 1, 3, and 7 post-infection). Before challenge and on days 2 and 6 post-infection, blood samples were taken from all piglets to evaluate serum level of prostaglandin E metabolite (PGEM), tumor-necrosis-factor- α (TNF- α) and interleukin-8 (IL-8). At days 3 and 4 post-infection, pigs from ATB group showed no diarrhea compared to pigs from CTRL and CFF groups. At days 4 post-infection, pigs from COL-CFF group showed no diarrhea compared to pigs from CTRL and CFF groups. Regarding fecal excretion at day 1 post-infection, in ATB group, pigs showed lower *Salmonella* fecal excretion than in CFF group and the lower weight piglets showed a higher fecal excretion than the higher weight piglets. The ATB group pigs showed a lower *Salmonella* fecal excretion level than pigs from CTRL group at 3 days post-infection. A significant time effect indicated that serum level of PGEM was significantly reduced 2 and 6 days post-infection in comparison to day 0 (before challenge) whereas TNF- α and IL-8 levels were significantly increased after *Salmonella* challenge.

Introduction

In our strategy to improve development and health of weaning pigs and to minimize incidence of diseases caused by different pathogens such as *Escherichia coli* (Fairbrother *et al.*, 2005) and *Salmonella* (Berger and Wierup, 2012), the effect of a combination of functional foods capable of modulating the development of systemic and mucosal immune system, physical barrier of the intestine and bacterial populations in the gut need to be further examined. Among functional foods, bovine milk by-products, including colostrum and whey, cranberry products, essential oil, probiotics, prebiotics, essential fatty acids and vitamins are considered as good candidates. For example, both colostrum and milk are rich in oligosaccharides and these molecules have the potential to inhibit binding of enteric pathogens to intestinal host cell receptors (Newburg *et al.*, 2005). Cranberry has been shown to have beneficial effects on health through their antioxidant property and antimicrobial activity against foodborne pathogens (Wu *et al.*, 2008). Recent study on essential oils as alternative to antibiotics have demonstrated that some essential oils demonstrated a good potential to antimicrobial activity towards bacterial pathogens found in pigs (including *S. Typhimurium*) (Si *et al.*, 2006). Probiotics are bacteria with beneficial effects on host gut health through immunomodulation (Lessard *et al.* 2009). In this study, we evaluated the impact of functional foods on *Salmonella* carriage in pigs, more specifically; we evaluated the presence of *Salmonella* in different organs, clinical signs and level of prostaglandin E metabolite (PGEM), tumor-necrosis-factor- α (TNF- α), and interleukin-8 (IL-8) in sera.

Material and Methods

***Salmonella* challenge strain dose and detection:** *Salmonella* Typhimurium DT-104 strain #4393 rifampicin resistant, isolated from a clinical case of salmonellosis (Côté *et al.* 2004), was used for inoculation. The isolate was thawed on nutrient agar and was grown in Buffered Peptone Water medium in a shaking incubator at 150 rpm, both at 37°C. The first part of the project was to determine the proper dose of *S. Typhimurium* needed to establish the carrier state in piglets and

to characterize the *Salmonella* infection kinetic. Three groups of 10 piglets were exposed to different oral doses (1×10^4 , 1×10^6 or 1×10^8 CFU) of *S. Typhimurium* strain #4393. The research of *Salmonella* in feces, mesenteric lymph nodes and junction ileo-cecal was carried out using a modification of ISO6579 2002(E): Annex D, using BGS agar with rifampicin as the single isolation medium.

Design of the study: At the Dairy and Swine R&D Center, 48 sows were inseminated to obtain 32 litters of piglets. Four farrowing periods were planned to do 4 experimental infections. At each farrowing period, 8/12 sows and their litter were chosen for the experimental infection. After 14 days of lactation, the 4 smaller and 4 bigger piglets were identified. At weaning (20-21 days-old), the 4 smaller piglets were put in the same pen and the 4 bigger piglets were in another pen. Then, they were assigned to 1 of the 4 feed additives as follow: 1- Basic diet + 3,5% plasma proteins (CTRL), 2- Basic diet + 3,5% plasma proteins + antibiotics (ATB), 3- Basic diet + 3,5% plasma proteins + cocktail of functional foods (cranberry extract, encapsulated calvacrol (kindly provided by Drs Wang and Gong, Guelph, AAFC), yeast extract, vitamins and mineral with organic Se, vitamins A, D and B, probiotic *Pediococcus acidilactici* MA18/5M) (CFF), and 4- Basic diet + bovine colostrum + cocktail of functional foods (COL-CFF).

Twenty-one days after weaning (day 42), all remaining piglets (2 smaller and 2 bigger piglets/litter) were transferred in level II facilities of the Veterinary School in St-Hyacinthe for 7 days of acclimation before the experiment. An individual clinical exam was done each day during the acclimation period: general state (score 1 to 6), respiration, body temperature, and consistency fecal score (0-3). Feces samples were collected 24 hrs after their arrival to be sure that piglets were *Salmonella* free before to start the experiment. One week after transfer (day 49), piglets were orally inoculated with *S. Typhimurium* DT-104 #4393 at 1×10^8 CFU, and an individual clinical exam was done twice a day as described above. Fecal samples were taken to evaluate the *Salmonella* post-infection shedding (days 1-3-7). On days 2 and 6 post-infection, blood samples were taken from all piglets to evaluate serum level of PGEM, TNF- α , and IL-8. Three and 7 days post-infection, two piglets per litter were euthanized (1 smaller/1 bigger). Ileo-cecal junction and mesenteric lymph nodes were collected to detect and quantify *Salmonella*.

Data analysis: Body temperatures and cytokines were analyzed using a repeated-measures linear model with treatment as a between-subject factor, time as a within-subject factor and block and id within the block as random factors. A priori contrasts were performed between pairs of means using the sequential Bonferroni procedure to adjust comparison-wise alpha levels. Categorical data (such as consistency fecal scores) were analyzed using the Cochran-Mantel-Haenszel test. An exact chi-square test was used to test the relationship between prevalence data in different compartments (feces, junction, and rectum) and treatment separately at each time period. Log10 transformed bacterial counts were analyzed using a mixed linear model with treatment as a fixed factor, id as a random factor and sex, weight and dose as co-factors. Tukey's post-hoc tests were used to compare pairs of treatment means. The level of statistical significance was set at 0.05 and analyses were carried out using SAS v.9.3 (Cary, N.C.).

Results

In the first part of the study, results showed that piglets from group 3 (1×10^8 CFU) demonstrated the higher level of carrier state during the *Salmonella* challenge and this dose was used for the second part of the study on the impact of functional foods on the course of *Salmonella* infection in piglets. All piglets were *Salmonella* free before to start the experiment. For the clinical exam, no significant observations were noted between the groups. The majority of animals presented a normal respiration and a general state score (1). The temperature varies significantly in the time ($p < 0.0001$) and it is similar from a group to other one, without any group effect. At days 3 and 4 post-infection, pigs from ATB group showed no diarrhea compared to pigs from CTRL and CFF groups. At days 4 post-infection, COL-CFF group pigs showed no diarrhea compared to pigs from CTRL and CFF groups. In a general way, the majority of piglets showed presence of *Salmonella* in feces, in mesenteric lymph nodes and in the ileo-cecal junction at days 1, 3 and 7 post-infection. Regarding fecal excretion level at day 1 post-infection, pigs from ATB group showed lower *Salmonella* fecal excretion level than CFF group ($P = 0.0494$) and the lower weight piglets showed a higher fecal excretion level than the higher weight piglets ($P = 0.0002$). The ATB group pigs showed a lower *Salmonella* fecal excretion level than CTRL group at 3 days post-infection ($p = 0.0157$).

Table 1: Effect of *Salmonella* infection on blood prostaglandin (PGEM), tumor-necrosis-factor- α (TNF- α) levels and interleukin-8 (IL-8).

Inflammatory Factor	Day 0	Day 2 post-infection	Day 6 post-infection	SEM	D0 vs. D2 (P value)	D2 vs. D6 (P value)
PGEM (pg/ml)	151.0	107.1	85.9	10.5	<0.001	<.0001
TNF- α (pg/ml)	44.4	59.7	60.1	4.9	<0.001	0.60
IL-8 (pg/ml)	238.2	225.8	323.45	42.0	0.20	0.02

At 7 days post-infection, a significant interaction ($p=0.0146$) was observed between the treatment and the weight on the quantification of *Salmonella* in the lymph nodes. The lower weight piglets in the ATB group pigs were more colonized by *Salmonella* in lymphatic nodes than the higher weigh piglets. The opposite scenario was observed for piglets in the CFF group pigs.

A significant time effect showed that PGEM level was significantly reduced 2 and 6 days post *Salmonella* challenge whereas TNF- α and IL-8 were respectively increased on day 2 and day 6 after *Salmonella* challenge (Table 1). Dietary treatments had no effect on PGEM, TNF- α , and IL-8 serum level.

Discussion

In the first part of this study, we developed a reproducible model for future studies on strategies to control *Salmonella* in pigs. Piglets infected with 1×10^8 CFU of *Salmonella* Typhimurium DT104 showed the highest level of carrier state during the *Salmonella* challenge. During protection assays, the use of colostrums and probiotics in a functional food (COL-CFF) showed a reduction of diarrhea and this observation is also seen with the ATB group. Bovine milk by-products have been demonstrated to contain bioactive molecules with immuno-regulatory and antimicrobial properties (Cross and Gill, 2000; Schlimme *et al.*, 2000). Both colostrum and milk are rich in oligosaccharides and there is evidence that neutral oligosaccharides present in milk are not digested or absorbed into the small intestine, but instead delivered into the colon (Boehm and Stahl, 2007). These molecules have been shown to play an important role in the establishment of different bacterial populations in the gut (Kuntz *et al.*, 2008) and have the potential to inhibit binding of pathogenic Gram-negative bacteria such as *E. coli* to intestinal host cells (Newburg *et al.* 2005). Because of all these functional properties, bovine colostrum could then be more appropriate than plasma protein as protein source in weanling diet.

In this study, *Salmonella* infection was efficient in modulating the release of inflammatory mediators such as PGEM, TNF- α and IL-8. Our results are in contradiction with another study from Balaji *et al* (2000) indicating that *Salmonella* infection did not alter plasma TNF α and PGE $_2$ in pigs. These authors hypothesized that PGE $_2$ released by inflamed gut tissue remain sequestered locally and does not contribute to elevate PGE $_2$ in blood (Balaji *et al.*, 2000). However, it has been reported that the clinical state of *Salmonella* orally infected pigs correlated with bacterial translocation and levels of the inflammatory cytokines IL-8 and TNF- α in plasma and intestinal lavages (Splichalova *et al.* 2011). In our study, it is also interesting to note that plasma level of TNF- α and IL-8 differently varied with the course of infection.

Conclusion

This study permit to determine the proper dose of *S. Typhimurium* DT104 needed to establish the carrier state in piglets and to characterize the *Salmonella* infection kinetic. This model is useful to evaluate the mechanisms of action of natural feed additives strategies as alternative to antibiotics to control *Salmonella* infection and at the end reduce risk of foodborne infection in human but more investigations are needed to adjust the feed treatment and optimize the efficacy.

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